ATTACHMENT II – PROTOCOL

Ecolab GLP Study Number:
2000034

REGULATED PESTICIDE EFFICACY STUDY PROTOCOL

STUDY TITLE: 920209 Soft Surface Sanitizing Efficacy

EPA REG. NO.: 1677-[pending]

ECOLAB GLP STUDY NUMBER: 2000034

STUDY OBJECTIVE

The purpose of this study is to evaluate the sanitizing efficacy of the test substance on soft surfaces following a modification of the current ASTM E1153 method. The test systems to be evaluated as well as the experimental design is described below.

Test Parameters	
Ecolab SOP Number:	MS120; *Soft Surface Sanitizer Method*
Test Systems:	*Staphylococcus aureus* ATCC 6538 *Klebsiella aerogenes* ATCC 13048
Organic Soil Load:	5% Fetal Bovine Serum
Exposure Time:	5 minutes
Exposure Temperature:	Ambient (15-30°C)
Test Substance Concentration:	4.25 oz/gallon so that the active ingredient is at or below the lower limit of 3131 ppm Dodecylbenzenesulfonic Acid.
Test Substance Diluent:	400 ppm AOAC Synthetic Hard Water
Test Surface:	1" x 1" synthetic fabric carriers (100% polyester)
Number of Test Carriers:	5
Neutralizing Medium:	Letheen Broth
Plating Medium:	Tryptic Soy Agar or Tryptic Soy Agar with 5% Sheep's Blood
Incubation of Test:	*Staphylococcus aureus* ATCC 6538: 48 ± 4 hours at 35 ± 2°C *Klebsiella aerogenes* ATCC 13048: 48 ± 4 hours at 30 ± 2°C

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TEST SUBSTANCE IDENTIFICATION

Test Substance Name: **920209**

Alternate Test Substance Name: VEGAS NONPEROX MLTSURF CL

Formula Code: 920209

Batch Identification & Date of Manufacture:

Batch Identification Number	Date of Manufacture
5439EG2200	October 28, 2019
5439EG2400	October 29, 2019
5439EG2600	October 30, 2019

Test Substance Stability & Characterization

The stability of the test substance was determined prior to or concurrent with this study under Ecolab GLP study number1900099.

The chemical quality of the test substance concentrate and at least one batch of test substance use-solution was/will be verified to be acceptable prior to use in this study under Ecolab GLP study number 1900113.

Initiation and termination dates are documented within the study referenced. A Certificate of Analysis for each batch of test substance will be appended to the final report.

Test Substance Concentration

Antimicrobial testing of the dilutable test substance will be performed with the test substance diluted at 4.25 oz/gallon to result in the active ingredient at or below the lower limit.

The ppm of active ingredient at the lower limit is determined by:

$$\text{ppm active at lower limit} = \left(\frac{\text{\% Active at LCL}}{100\%}\right)\left(\frac{\text{\% Dilution}}{100\%}\right)\left(\text{Specific Gravity} \times 10^6\right)$$

where:

$$\text{\% Dilution} = \left(\frac{\text{x oz.}}{\text{x gallon}}\right)\left(\frac{\text{1 gallon}}{\text{128 oz.}}\right)(100\%)$$

Active Ingredient	% Active at LCL from CSF	Percent Dilution	Specific Gravity	ppm active at LCL
Dodecylbenzenesulfonic Acid	9.2%	3.320%	1.025	3131

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The target mass of the product to achieve the desired concentration for a 200 g solution was determined as follows:

$$\text{Target mass (g) of product} = \frac{(\text{ppm Active at LCL})(\text{Total mass of use-solution})(100\%)}{(10^6)(\%\ \text{Active Ingredient Result})}$$

Dilution procedure for efficacy testing (or equivalent dilution):

Test Substance Batch Identification	Active Ingredient	Actual Active Ingredient Result	Target Mass of Test Substance (±0.03g)	Target Mass of Diluent (±0.03g)
5439EG2200	Dodecylbenzenesulfonic Acid	9.9%	6.33 g	193.67 g
5439EG2400	Dodecylbenzenesulfonic Acid	9.9%	6.33 g	193.67 g
5439EG2600	Dodecylbenzenesulfonic Acid	10.0%	6.26 g	193.74 g

PESTICIDE EFFICACY EXPERIMENTAL DESIGN

The following are proposed experimental start and termination dates:

Experimental Start Date: March 2020
Experimental Termination Date: March 2020

Test Methods

Pesticide efficacy data will be generated by the Microbiology Lab using the most current version of the Standard Operating Procedures (SOP) listed below. The SOP version followed in testing will be documented in the raw data and a copy of the SOP will be retained in the study file.

SOP Number	Method Name
MS008	*Synthetic Hard Water Preparation & Standardization*
MS120	*Soft Surface Sanitizer Method*

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Test Method Summary:

According to MS120, scoured fabric test carriers are inoculated with the test system and allowed to dry creating a film. The dried, inoculated carriers are exposed to 5 mL of the test substance prepared at the desired concentration. Following a prescribed exposure time, an appropriate neutralizer is added to the treatment vessel and the carriers are mixed. Appropriate aliquots of the neutralized material are plated onto agar medium to enumerate the survivors.

Appropriate study controls are conducted according to the test method SOP to evaluate the inoculum count, test square inoculum count, to confirm neutralization, to confirm test system purity, and to appropriately evaluate sterility of the media and/or reagents used.

Test Method Modifications: None

Test Method Requirement and Test System Justification

The test methods selected are based on a modification of the current ASTM E1153 method and the corresponding test method requirements have been determined based on U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP) Product Performance Test Guidelines 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides (February 2018) and 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles – Efficacy Data Recommendations (December 2012).

The test system(s) used to support sanitizer claims have been selected based on OCSPP Product Performance Test Guideline 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles – Efficacy Data Recommendations (December 2012).

Test System Identification

Each test system selected for this study will be identified by Gram stain and by observing the colony morphology for conformity to expected morphology.

Test System Source: American Type Culture Collection (ATCC), Manassas, VA or an alternative reputable source

The actual source will be reported.

Interpretation of Test Results

The performance standard for a soft surface sanitizer is $\geq$ 99.9% reduction of the test system as compared to the numbers control results.

Statement of Proposed Statistical Method & Method for Control of Bias

None

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PROPOSED QUALITY ASSURANCE UNIT (QAU) MONITORING

Ecolab QAU inspections, (e.g., protocol audits, critical phase audits and final report audits), that are performed along with their dates, the date reported to the study director and management, and auditor names will be included in the study final report.

DATA RETENTION

Following the completion of the study, the original final report and raw data will be transferred to Ecolab Archives located at the Ecolab Schuman Campus in Eagan, MN or at an approved off-site location. All records that would be required to reconstruct the study and demonstrate adherence to the protocol will be maintained for the life of the commercial product plus four years.

TEST SUBSTANCE RETENTION

An aliquot of each batch of test substance will be retained in the appropriate sample retention cabinet in the restricted access storage room for regulated samples at the Ecolab Schuman Campus in Eagan, MN and will be retained for at least five years after the manufacture/preparation date. Samples of relatively fragile test substances will be retained only as long as the quality of the preparation affords evaluation. Test substance not dispersed for retention or efficacy testing, as applicable, will be stored in Ecolab Microbiological Services cabinet until disposed of.

GOOD LABORATORY PRACTICES

This study will be conducted according to Good Laboratory Practices, as stated in 40 CFR Part 160. If it becomes necessary to make changes in the approved protocol, the revisions and reasons for change will be documented, reported to the sponsor and will become part of the permanent file for that study. The sponsor will be notified as soon as it is practical whenever an event occurs that could have an effect on the validity of the study.

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- **Name and Address of Sponsor**

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Sponsor — Date: 03/20/2020

Study Director — Date: 3/31/2020